PATENT
Attorney Docket No.: 02558P-001340US

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of:

James Blake *et al.*

Application No.: 09/733,239

Filed: December 8, 2000

For: CYSTEINE THIOL-PROTECTED PEPTIDES FOR USE IN IMMUNOASSAYS

Customer No.: 20350

Confirmation No. 1694

Examiner: Emily M. Le

Technology Center/Art Unit: 1648

DECLARATION OF PATRICK COLEMAN UNDER 37 C.F.R. § 1.132

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

I, Patrick Coleman, declare and state as follows:

1. I am a co-inventor of the subject matter of U.S. Patent Application No. 09/733,239, entitled "Cysteine thiol-protected peptides for use in immunoassays" (hereinafter the '239 application" or "the application").

2. I currently hold the position of Manager, Biologics, R&D and Manufacturing at Redmond Operations, Bio-Rad Laboratories. I have been employed with Bio-Rad Laboratories since 1999. Before my employment with Bio-Rad Laboratories, from 1992 to 1999, I was employed as Director of Research and Development with Sanofi Diagnostics Pasteur and Genetic Systems Corporation, the original assignee of the applications to which the '239 application claims priority, including U.S. Patent Application 140,696, now U.S. Patent No. 5,439,792, of which I am also a co-inventor. I have a Ph.D. in Biochemistry from Stanford University. I have over 30 years of post-graduate scientific experience, including, for example,

in the areas of biochemistry, molecular biology, and development of synthetic peptides for immunodiagnostics. A copy of curriculum vitae is attached hereto as Exhibit 1.

3. As a co-inventor of the subject matter described in the '239 application, and as a researcher in the fields of biochemistry, molecular biology, and immunodiagnostics development with over 30 years of post-graduate experience (*see* ¶ 2), I am a person of skill in the art to which the invention as claimed in the application pertains.

4. I have read the Office Action dated May 31, 2006 ("Office Action") issued by Examiner Le.

5. I understand from the Office Action that pending claims 1-3, 7-9, 48, and 49 stand rejected as allegedly obvious over Cosand *et al.* (U.S. Patent No. 4,629,783; hereinafter "Cosand '783") in view of Rosen *et al.* (WO 87/06005; hereinafter "Rosen") and Storey *et al.* (*J. Am. Chem. Soc.* 94:6170-6178, 1972; hereinafter "Storey"). I also understand that pending claims 1, 4-6, and 8-13 stand rejected as allegedly obvious over the above-cited references in further view of Cosand *et al.* (U.S. Patent No. 5,075,211; hereinafter "Cosand '211").

6. I have read and understand the documents referenced in ¶ 5 above.

7. The statements set forth herein are offered to address the Examiner's remarks in the Office Action and to show that, as of the effective filing date of the '239 application, the references discussed in the Office Action would not have led an artisan of ordinary skill to the invention as presently claimed in the application.

8. I understand that independent claim 1 of the '239 application reads as follows:

> 1. (Previously presented) A composition comprising an isolated peptide, wherein said peptide is immobilized on a solid phase following synthesis of the peptide on a synthesis solid support and cleavage of the peptide therefrom, said peptide having at least one epitope capable of binding antibodies to a protein comprising the epitope(s),

wherein the peptide comprises an amino acid sequence of six to 50 amino acids, and the sequence comprises two Cys residues which are separated from each other by at least two but fewer than twenty non-Cys amino acid residues and wherein thiol groups of the Cys residues are reversibly protected from oxidation by a chemically reversible means resistant to highly acidic cleavage conditions used for peptide cleavage from the synthesis solid support.

9. I understand that the Examiner cites to Cosand '783 as teaching "peptide V", comprising two Cys residues that are separated by 5 non-Cys residues and having a sequence identical to SEQ ID NO:1 of the '239 application. (*See* Office Action at p. 5, last para. bridging to p. 6.) The 26 amino acid residues of peptide V correspond to a part of the HIV gp41 glycoprotein.

10. I understand that the Examiner cites to Rosen as teaching that "peptides that possess more than two cysteine residues may form cyclic monomers, linear or cyclic dimers, and linear polymers of various lengths due to the reduction of the thiol group present on cysteine residues by oxidation." (Office Action at p. 6, 3rd para., citing Rosen at p. 10, ll. 15-30.) Rosen is also cited in the Office Action as noting that "the cyclic monomer of the peptides is believed to be less efficient in binding to the microtiter wells, and is less suited as a solid phase component of the [HIV ELISA]." (*Id.* at p. 6, last para. bridging to p. 7, citing Rosen at p. 22, ll. 27-35.)

11. I also understand that the Examiner cites to Storey as discussing the use of "ethylcarbamoyl to protect cysteine from the highly acidic … condition used during cleavage of the peptide from its synthesis solid support." (Office Action at p. 7, 1st para.)

12. I understand that, based on the Examiner's characterization of the cited references as summarized above, the Examiner asserts that one of ordinary skill in the art would have been motivated to "use ethylcarbamoyl to reversibly protect cysteine from oxidation" so as to "control the oxidative form of peptides comprising more than one cysteine residue, and to provide a suitable solid phase component of the [ELISA]." (Office Action at p. 7, 1st full para.)

13. For reasons further set forth herein, Cosand '783, Rosen, and Storey would not have led a skilled artisan to the peptide as presently recited in claim 1. None of these references, whether considered alone or in combination, teach or suggest the present invention's solution to a technical problem in the immunoassay art, nor otherwise teach or suggest a cysteine-containing, immobilized peptide as presently claimed in the application.

14. First, the peptide composition as recited in claim 1 of the '239 application solves a technical problem in the area of immunoassay design. In particular, the oxidative form of a cysteine-containing peptide can influence antigenicity of the peptide when used in solid-phase immunoassays. The present invention, as claimed in the application, allows for control over the oxidative form of a cysteine-containing peptide immobilized on a solid phase after cleavage and purification. The '239 application states, *inter alia*, as follows regarding the technical problem addressed by the present invention:

> The presence of cysteine residues in a peptide allows for the formation of inter- and intra-molecular disulfide bonds during purification, immobilization, and upon long term storage of a deprotected peptide. Therefore, such peptide compositions are usually immobilized on a solid phase as a mixture of a variety of oxidative forms, including monomer, dimer and polymers of various sizes. Precautions are generally not taken to control the oxidative form of peptides immobilized on a solid phase.... The variety of oxidative forms of the peptides may be a source of variability in sensitive immunoassay and this may also influence the results based on those assays.
>
> ['239 application at p. 3, ll. 19-28, and p. 4, ll. 1-4.]

15. As further noted in the application, and with particular regard to immunoreactivity, the formation of intramolecular (rather than intermolecular) disulfide bonds can contribute to the conformation of the peptide capable of binding antibodies engendered by the native protein. (*See id.* at p. 8, ll. 27-31.)

16. Because an unprotected, cysteine-containing peptide will also form dimeric and polymeric oxidative forms, however, the proportion of the monomeric, intramolecularly disulfide-bonded ("cyclic") forms in a peptide mixture will be substantially lower than can be achieved with immobilized, reversibly protected peptides, which can be subjected to specific oxidation conditions in a more controlled manner.

17. Thus, hindering development of oxidative forms during cleavage and processing of the peptide can allow for improved peptide immunological reactivity. (*See, e.g., id.* at p. 8, ll. 18-23.)

18. Cosand '783 does not teach or suggest, nor is Cosand '783 otherwise concerned with, controlling the oxidative form of a cysteine-containing peptide. Cosand discloses new (gag and env) peptide compositions, including, *e.g.*, cysteine containing peptide 39 (peptide V). Cosand '783, however, does not discuss the various oxidative forms of the disclosed peptides and the influence of such oxidative forms in immunological assays.

19. Indeed, in regard to the use of the disclosed peptides in HIV immunoassays, Cosand '783 shows peptide V, when immobilized, to be very reactive with LAV antibodies. (*See* Cosand '783 at Tables I and III.) Cosand '783 does not discuss any deficiencies of peptide V, or any other cysteine-containing peptide, that would specifically suggest to the skilled artisan to, for example, reversibly protect a cysteine residue during the peptide's cleavage, purification, and immobilization on a solid phase for use in immunoassays. Thus, the skilled artisan reading Cosand '783 would understand the immunoassay methods as disclosed therein to be specific and self-sufficient.

20. For the reasons summarized above in ¶¶ 18 and 19, Cosand does not provide any clear and particular motivation to modify the peptides disclosed therein in a manner that would lead to the presently claimed invention.

21. In the case of Rosen, although this reference briefly discusses the presence of various oxidative forms in immobilized, cysteine-containing peptides, Rosen does not provide a solution. In fact, Rosen actually teaches away from the present invention by teaching that

polymeric oxidative forms are advantageous over the cyclic, monomeric forms. In this respect, Rosen states as follows:

> The cyclic monomer form, while retaining a portion of the antigenicity of the polymer form, is believed to be less efficient in binding to the microtiter wells and is less suited as the solid phase component of the ELISA.
>
> [Rosen at p. 22, ll. 27-31.]

22. In view of the above, Rosen does not provide any clear and particular motivation to the skilled artisan to modify Cosand '783, in the manner proposed by the Examiner, so as to achieve the claimed invention. To the contrary, Rosen would instead suggest to the skilled artisan that the proportion of the polymeric oxidative form should be increased, which could not be achieved by reversibly protecting a cysteine-containing peptide. The skilled artisan reading Rosen would thus be led to maintain the peptide in a deprotected state to facilitate formation of the polymeric oxidative form prior to immobilization, and further to control the proportion of the polymeric form by adjusting the oxidative conditions with respect to temperature, pH, peptide concentration, and the like, as explicitly suggested by Rosen (*see* Rosen at p. 22, l. 35, to p. 23, l. 4).

23. Storey does not remedy the deficiencies of Cosand and Rosen as discussed above. Storey is an academic, organic-chemistry-oriented article concerned with synthesizing polypeptides, particularly polypeptides derived from a ribonuclease of about 80 amino acids in length, the polypeptides being constructed from separately synthesized, protected peptides. (*See* Storey at, *e.g.*, Abstract.) Storey does <u>not</u> discuss, however, the influence of reversibly protecting cysteine thiol groups on antigenicity, nor does Storey discuss the diagnostic importance of controlling disulfide bond formation when preparing peptide reagents for immunoassay. Storey does not discuss or otherwise suggest that using the S-ethylcarbamoyl group would be useful or advantageous in the synthesis of a peptide for use as an antigen.

24. For the reasons summarized above in ¶ 23, Storey does not provide any clear and particular motivation to the skilled artisan to modify the peptide of Cosand '783 in the manner proposed by the Examiner, so as to achieve the presently claimed invention.

25. With regard to Cosand '211, as cited against claims 1, 4-6, and 8-13, Cosand '211 also does not remedy the deficiencies of Cosand '783, Rosen, and Storey. I understand that Cosand '211 is cited by the Examiner as teaching, *inter alia*, the "use of cysteine in combination of other intervening amino acid spacers." (Office Action at p. 8, last para.) Cosand '211 does not discuss or otherwise suggest, however, the influence of reversibly protecting cysteine thiol groups on antigenicity, nor does Cosand '211 discuss the diagnostic importance of keeping cysteine thiol groups protected when preparing peptide reagents for immunoassay. Therefore, Cosand '211 does not provide any clear and particular motiviation to modify the peptide of Cosand '783 so as to achieve the presently claimed invention.

26. In addition to the lack of any teaching or suggestion of the presently claimed invention in the references cited in the Office Action, the presently claimed invention has led to the commercial success of immunoassay products manufactured and distributed by Bio-Rad Laboratories ("Bio-Rad") , the assignee of the '239 application, as set forth further below.

27. During my employment with Genetic Systems Corporation and Bio-Rad Laboratories, I have been involved with the development of several diagnostic immunoassay products.

28. As a co-inventor of the '239 application and its priority applications, and as a person of skill in the art directly involved with research and development of diagnostic immunoassay products, I am familiar not only with the technical features of these immunoassay products, but also with how these technical features relate to the presently claimed invention and the commercial success of these products in the marketplace.

29. Bio-Rad's immunoassay products include two blood screening products, licensed by the United States Food and Drug Administration ("FDA"), for detection of HIV that

employ peptides as presently claimed in the '239 application. These products are the HIV-1/HIV-2 Synthetic Peptide EIA ("Peptide EIA") and the Multispot HIV-1/HIV2 Rapid Test ("Rapid Test").

30. The Peptide EIA was licensed by the FDA in August, 1997, and still remains on the market today. This product has been used to screen blood and plasma for the presence of HIV antibodies since its approval. It has been one of two FDA-licensed assays used to safeguard the U.S. blood/plasma supply over the last nine years. Over this period, nearly 100 million tests have been sold, with a total sales revenue of over $100 million. By any standard in the industry, the Peptide EIA has been commercially successful, and an important product in the U.S. marketplace.

31. The Rapid Test is designed to diagnose HIV infection and, in particular, to differentiate between an HIV-1 infection and an HIV-2 infection. It is run in a totally manual mode and is not intended for any high volume blood screening application. The Rapid Test can be run in a field hospital as well as in a standard laboratory. At the time the Rapid Test was first developed, because the FDA was not ready to approve any rapid HIV test, the Rapid Test was initially not approved by FDA for sale and use in the U.S. Instead, this technology was transferred to France, where it was manufactured, and the kits were sold in many countries, especially in African nations. After several years, the production of the Rapid Test kits was transferred back to the U.S. and it has been approved by the FDA for U.S. distribution. To date, the Rapid Test is the only commercial product for the detection and differentiation of HIV-1 and HIV-2 infections. This product has had a notable world-wide reputation, and it has been used and promoted by the Centers for Disease Control (CDC). The Rapid Test has been extremely useful in tracking the incidence of HIV-2 infections in the U.S. population. Although its usage volume is expectedly much lower and it is not intended for blood screening, total sales revenue in France is approximately $10 million, and, since its entry into the U.S. marketplace in 2005, total U.S. sales revenue is nearly $3 million. Thus, the Rapid Test has also been commercially successful and an important product in the marketplace.

32. Both the Peptide EIA and the Rapid Test have high sensitivity and produce highly accurate results, which have contributed to their success in the HIV field.

33. The high sensitivity and accuracy of the Peptide EIA and Rapid Test can be attributed to, *inter alia*, the technology as presently embodied in claim 1 of the '239 application, as discussed further below.

34. Each of the Peptide EIA and Rapid Test employ, *inter alia*, peptides having an amino acid sequence of six to 50 amino acids, the sequence comprising two cysteine residues which are separated from each other by at least two but fewer than twenty non-cysteine amino acid residues. These peptides include peptides derived from the HIV transmembrane proteins HIV-1 gp41 and HIV-2 gp36. The peptides range in size from 17 amino acid residues to 35 amino acid residues and include native cysteines separated by 5 amino acid residues.

35. During synthesis of these peptides (*see* ¶ 34) on a synthesis solid support, as well as during subsequent cleavage, the native cysteines are reversibly protected from oxidation with an ethylcarbamoyl (EC) group, which is a chemically reversible protection means resistant to the highly acidic conditions used to cleave the peptides from the synthesis solid support.

36. The use of the chemically reversible protection means, as discussed above in ¶ 35, allows the oxidative state of the peptide to be controlled during formation of the immunoassay solid phase. The cysteines are prevented from forming the cyclic, epitope structure, until after the peptides are immobilized on their respective solid phases. In particular, because the cysteine thiols have been protected during synthesis, cleavage, and purification, polymeric oxidative forms of the peptides are substantially minimized. Once immobilized on the immunoassay solid phase, deprotection of the peptide, together with the application of appropriate oxidation conditions, allows the peptide to be maintained in a purified, cyclic (intramolecularly disulfide-bonded) form.

37. It is the purified, cyclic form of these peptides (employed in the Peptide EIA and Rapid Test) that has improved immunological reactivity over other oxidative forms. In particular, it is the purified, cyclic form of the peptides that confers much of the high specificity and sensitivity of the Peptide EIA and Rapid Test, as well as the accuracy of the results. Immune recognition of the immobilized peptides, by natural HIV antibodies, is poor or absent prior to the removal of the EC groups. After removal of the protecting groups, cyclization of the cysteine loops, and dimer formation, the specific immune recognition increases dramatically. This demonstration of the sensitivity to the detection of HIV antibodies was critical for achieving FDA approval and, as previously indicated above, the high sensitivity and accuracy of these immunoassays has contributed to their success in the HIV field.

38. Furthermore, preventing the formation of disulfide bonds during cleavage from the synthesis solid phase, and subsequent handling of the peptides during kit manufacturing operations, was also critical for meeting necessary FDA license criteria for peptide characterization. To receive FDA approval for the HIV peptides used in the Peptide EIA and Rapid Test, it was necessary to demonstrate the consistency and purity of the peptides, including these properties *vis-à-vis* their oxidative state once immobilized on the solid phase. Such consistency and purity was achieved by preventing random, sulfhydryl redox chemistry during peptide cleavage and subsequent handling, as discussed above. To create a manufacturable solid phase, controlled deprotection of the reversible blocking groups was employed once the peptides were applied to the microplate or latex bead solid phases. Thus, peptide compositions as presently embodied in the '239 claims were important in leading to FDA approval of the Peptide EIA and Rapid Test immunoassays, and as well as their commercial success.

39. I further declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that I make these statements with the knowledge that willful false statements and the like are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize validity of the application or any patent issuing therefrom.

Executed this 22nd day of November, 2006 By: Patrick J. Coleman

Name: Patrick Coleman

Title: Manager Biologics R&D and Manufacturing